OXYGEN DEFICIENCY AND SEWAGE PROTOZOA: WITH DESCRIPTIONS OF SOME NEW SPECIES [1]

JAMES B. LACKEY

(From the Sewage Investigations Laboratory, New Brunswick, N. J., and Southwestern College, Memphis, Tennessee)

It is a common observation that the protozoa of hay infusions and other liquid media generally seek the top of the culture where oxygen is to be found. Investigation of a rich culture either in a jar or on a slide shows that only a few inhabit the oxygen-poor regions: *Metopus* among the ciliates; some of the very small flagellates and small amoebas, mostly of the limax type. Anaërobic protozoa are well known. Juday (1919) has described a freshwater anaërobic ciliate; Lauterborn (1908) has discussed a number of species, many of them bizarre forms, from the oxygen-poor waters of the Rhine. Lackey (1925, 1926) has listed twenty-nine species common in sewage containing little or no dissolved oxygen, but often abundant H_2S or CO_2; while Cole (1921) has discussed the oxygen supply of animals living under such conditions.

Most of the protozoa inhabiting sludge tanks are small and do not occur in great numbers—except those which live at the very top and are not considered in this paper, for they have an abundance of oxygen and may be transient forms in the tanks as well—so that they are not often noted by students unless a careful search is made. All of those ciliates characterized by Noland (1925) as living in water whose oxygen saturation is below 45 per cent have been encountered at one time or another, but usually in small numbers, with the exception of *Metopus sigmoides*. As a result of examining the protozoan fauna of the digestion tanks of five New Jersey towns, the Passaic River in some of its most polluted stretches, and septic tanks from three locations in Tennessee, it has been found that the same small group of protozoa is to be found in each of these situations.

Of the forms listed previously (Lackey, 1925) two ciliates and one flagellate were described as new species. The present paper includes four new flagellates and two new rhizopods. The wide distribution of this group argues that the factors limiting their occurrence are very largely those of anaërobism, for such conditions as pH, temperature, dissolved gases, and food substances must have varied widely in the

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several locations examined. Sufficient check has been made on the first two of these conditions in sludge digestion to make this assumption plausible, and it is known that they certainly vary from time to time, and that the amounts of CO_2 and H_2S fluctuate considerably also in any given tank, apparently without a corresponding fluctuation of the protozoan fauna as a whole, in the same tank. It is also certain that while all sewage contains decomposition products which can possibly serve as food for the organisms, the variety of these substances is too great to be a probable limiting factor.

To ascertain the effects of dissolved oxygen on two protozoa characteristic of sewage disposal plants, tall cylinders were set up, so that compressed air could be forced through porous plates in their bases. This allowed aëration of the sewage (eventually producing activated sludge) or kept the sewage under completely anaërobic conditions. *Opercularia* sp., a large peritrichous holozoic ciliate and an active bacterial feeder, was selected as the obligatory aërobe, for it is abundant in the trickling filters, but never occurs in its active state in the tanks. *Trepomonas* was selected as the obligatory anaërobe, for it is characteristic of the depths of the tanks. Table I shows the behavior of these two forms in the cylinders under conditions varying from no aëration to constant aëration. Preliminary examination of the sewage at the treatment plant showed 3300 *Trepomonas* and 500 *Opercularia* per cc., and after standing in a refrigerator 24 hours there were 2900 *Trepomonas* per cc., and no active Opercularia. It will be noted that the sludge when examined at the disposal plant contained both active *Opercularia* and *Trepomonas*. There was only a trace of oxygen present. The sample was taken to the laboratory and put in the refrigerator and 24 hours later contained no dissolved oxygen. Its protozoan population had changed in this time; all colonies of *Opercularia* contained only closed-up individuals.

After 6 hours aëration 65 per cent of the *Opercularia* were active, whereas with no aëration none of the *Opercularia* were active. No count was made, but the normal population of *Trepomonas* seemed to be present. Under aërobic conditions, however, the 6 hours had reduced the numbers of *Trepomonas* almost 96 per cent. Many dead or apparently dead ones were seen. Their protoplasm was greatly vacuolated and the cells sometimes much above normal size.

Twenty-four hours of aëration was sufficient to cause them to disappear and they never reappeared in this sample. All the *Opercularia*, on the contrary, became active and showed an increase in numbers for 24 hours, then a gradual decrease. When the experiment was discontinued after 196 hours, there were few protozoa of any sort in either aërated or unaërated sewage, but most of the *Opercularia* were active in the aërated, although they presented a rather starved appearance, due probably to disappearance of their food.

Although the results practically speak for themselves, supporting evidence is easily obtained. During an examination of settled sludge from the Chatham, N. J., activated sludge plant, on August 2, the sampling showed no dissolved oxygen and there were present 9200

TABLE I

Numbers of Organisms Present With and Without Aëration
(Numbers refer to active organisms throughout)

TIME	AËRATED		UN-AËRATED
		No. per cc.	No. per cc.
After 6 hours	Trepomonas	140	No count
	Opercularia	3200	0
After 24 hours	Trepomonas	0	3300
	Opercularia	12600	0
After 48 hours	Trepomonas	0	4500
	Opercularia	9300	0
After 120 hours	Trepomonas	0	5400
	Opercularia	8300	0
After 148 hours	Trepomonas	0	4000
	Opercularia	4500	0
After 172 hours	Trepomonas	0	2000
	Opercularia	4500	0

Trepomonas per cc., and only about 260 *Opercularia* per cc. The *Opercularia* were all closed up, inactive, while the *Trepomonas* were swimming about vigorously. This sample was then aërated for 3 hours, when an examination showed that all the *Opercularia* were actively feeding while about half the *Trepomonas* were killed and half active.

At the end of 6 hours aëration there were no signs of living *Trepomonas* in this cylinder, although the *Opercularia* were thriving and numerous small flagellates such as *Bodo, Cercobodo, Pleuromonas, Monas,* and *Tetramitus* were active. After 49 hours aëration a large protozoan fauna was present, the small flagellates of the group listed above being 15,300 per cc., and *Trepomonas* being absent. In a control of unaërated sludge there were, per cc., 5900 *Trepomonas*, 900 *Tetramitus*, active, and perhaps 175 inactive *Opercularia*.

It is not intended to list the protozoa occurring in activated sludge or trickling filters or, in general, in sewage which contains much dissolved oxygen. But from the above experiments and similar ones, it is apparent that many protozoa encyst when swept into the oxygen-free water of a digestion tank or the bottom of a river heavily polluted with sewage; that like *Opercularia*, they are able to reduce their metabolic activities to a minimum and so survive for a time, at least, in this adverse environment. If conditions become favorable again, they excyst and become active. It is also seen that there are some forms which live under either condition and some which thrive only in the absence of oxygen. This latter factor is so sharply limiting that we find a small and cosmopolitan group of protozoa which may be regarded as common in Imhoff tanks, septic tanks, and the deeper waters of heavily polluted streams.

Table II does not include many forms which have been seen, but not sufficiently studied for proper identification. Some of these will undoubtedly prove to be new species when better known. It is therefore seen that careful investigation of this particular type of habitat will be productive of an acquaintance with a decidedly unusual and interesting group of protozoa.

Description of New Species

The following descriptions are concerned with two new rhizopods and four new flagellates, which have been found in studying the waters of sewage disposal plants and the water of a creek near Camden, N. J., which was heavily polluted with sewage. Only one of the flagellates, *Chroomonas cyaneus*, was found in the creek, the others being from sewage disposal plants. The new species are illustrated in Plate I, enlarged about 1000 times.

VAHLKAMPFIA FRAGILIS, Sp. Nov. Fig. 4.

Organism small, free-living, 5 to 15 microns long, naked, relatively abundant in some sewage, especially in cultures. Pseudopodia lobose, two to three in number, clear, steadily formed rather rapidly and always at end of cell opposite contractile vacuole and nucleus.

One contractile vacuole, always posterior to the nucleus, constantly present, emptying seldom. Nucleus always oval, flattened, with two to three large endosomal granules (chromatin masses?) in center. Nuclear membrane delicate. Several hyaline spheres of varying size, oil or albumen, located in posterior region. Endoplasm finely granular, never extending into pseudopodia.

No flagellated stage found.

TABLE II

Group I

Present Only in Small Numbers or Infrequency in Digestion Tanks

RHIZOPODA	
Dimastigamoeba gruberi	Hartmanella hyalina
Vahlkampfia limax	Vahlkampfia albida
Vahlkampfia guttula	Chlamydophrys stercorea
FLAGELLATA	
Mastigella simplex	Dinomonas vorax
Cercobodo longicauda	Tetramitus descissus
Cercobodo crassicauda	Tetramitus pyriformis
Cercobodo ovatus	Hexamitus inflatus
Monas amoebina	Clautriavia parva
Monas minima	Euglena gracilis
Monas vulgaris	Menoidium incurvuum
Anthophysa vegetans	Peranema trichophorum
Helkesimastix faecicola	Distigma proteus
Bodo caudatus	Petalomonas mediocanellata
Bodo lens	Petalomonas carinata
Bodo mutabilis	Heteronema acus
Pleuromonas jaculans	Entosiphon sulcatum
Oicomonas termo	Notosolenus orbicularis
Cyathomonas truncata	Chilomonas paramecium
CILIATA	
Hexotrichia caudatum	Colpoda cucullus
Colpoda inflata	Cyclidium glaucoma
Glaucoma scintillans	Paramecium putrinum
Plagiopyla nasuta	

Group II

Always Present in Absence of Oxygen in Tanks

RHIZOPODA	
Chlamydophrys minor, sp. nov.	Vahlkampfia fragilis, sp. nov.
FLAGELLATA	
Mastigamoeba viridis	Mastigamoeba radiosa, sp. nov.
Mastigamoeba longifilum	Trepomonas agilis
Mastigamoeba reptans	Bodo glissans, sp. nov.
CILIATA	
Holophrya sp.	Saprodinium putrinum
Metopus sigmoides	Trimyema compressa

Nutrition apparently saprozoic.

Division binary in active stage. Forms very small cysts, thick-walled, with a few slight protuberances. Excysts in fresh raw sewage (by putting cover-slips in Petri dishes of old cultures, cysts are collected which can be watched on being transferred to fresh material).

Rather common in five samples obtained.

MASTIGELLA RADIOSA, Sp. Nov. Fig. 2.

Animal with spherical body, about 20 microns in diameter, free-swimming or floating. Pseudopodia much like those of *Amoeba radiosa,* occasionally branching, clear, up to 80 microns long. Single flagellum up to 100 microns long, used with a lashing or whiplike motion. One contractile vacuole. Nucleus central, spherical with endosome, membrane very thin, chromatin granules very small. Cytoplasm finely granular, no zones being present. Several types of granules such as oil spheres, or crystalline bodies, present.

Some apparent binary fission stages seen. Nutrition saprozoic. Rather rare; found in two of the tanks examined.

MASTIGAMOEBA VOLUTANS, Sp. Nov. Fig. 1.

Body elongate, flattened, 25 to 30 microns long, with a flagellum, vibratile in anterior third, slightly tapering, one and a half to two times body length. Cortical layer of protoplasm clear, hyaline, endoplasmic portion finely granular, reticulate, with numerous small, square crystals and numerous spheres of varying size. Due to the fact that they stain with methylene blue and that in some of these animals a clear vacuole was found which contained from one to three of these spheres, they are assumed to be volutin.

Pseudopodia very short, numerous, rounded.

Contractile vacuole single, in posterior part of cell.

Nucleus near anterior tip of cell, round in outline, with a central endosome surrounded by minute chromatin granules within a clear zone of nuclear sap. Membrane thin, with a rhizoplast visible in the living animal, from the flagellar insertion to its surface.

Nutrition apparently saprozoic.

Reproduction not observed.

Somewhat common in samples from six localities.

CHLAMYDOPHRYS MINOR, Sp. Nov. Fig. 3.

A very small form, showing constant differences from *C. stercorea.* Diameter of the transparent shell is 20 microns. Cytoplasm finely granular, extending out as a few finely-branching pseudopodia, which only occasionally anastomose and never become thread-like.

Contractile vacuole single, median. Nucleus centrally located, spherical, with a single endosome. A central zone of black, refringent, crescent-shaped granules is present.

Nutrition apparently saprozoic. Reproduction not observed. Movement slow.

Rather common in most sewage.

BODO GLISSANS, Sp. Nov. Fig. 6.

A somewhat flattened elongate organism, rarely over 20 microns long, cell of definite shape, but quite plastic. Two flagella, emerging on the ventral surface, anteriorly, from a lip-like fold. Swimming flagellum about body length, very tenuous and very active. Trailing flagellum slightly longer, thicker, used as an axis on which the animal glides. Forward movement rapid, path straight. When the animal changes its path, its amoeboid nature becomes evident.

No nucleus visible either in living specimens or those treated with neutral red or iodine. Some visible granules may be chromidia. One contractile vacuole, median. Protoplasm in anterior end mostly clear, but a few small spheres, probably oil, may be seen posteriorly. No visible kinetic apparatus. Reproduction by longitudinal division, while active. Nutrition saprophytic. No cysts observed. Rather common in sewage from several locations.

The animal is placed in the genus *Bodo*, following Calkins' (1926) key, but it is not holozoic, and the two flagella are not terminal but subterminal. None of the species described in Pascher's manual (1914) fit it. The position of the contractile vacuole and the inability to find an organized nucleus are also unique features.

CHROOMONAS CYANEUS, Sp. Nov. Fig. 5.

Organism pyriform, obliquely truncated anteriorly, tapering to a somewhat extended point posteriorly, never exceeding 10 microns in length. Two equal or subequal flagella emerge from a slight depression anteriorly. They are about body length, directed forward in swimming, and the path of the animal is a spiral. Two or more small contractile vacuoles are located at their base. There is no eyespot, nor can other structures be distinguished in this region. The nucleus is central and apparently there is no endosome, but only granular masses of chromatin. Chromatophores two in number, band-shaped, curved, of a bright blue color which speedily diffuses into the surrounding water when the animal disintegrates. Pellicle thin, rigid. One case of binary fission while active was noted, but no cysts were found.

These organisms were not found in sewage, but were abundant in the waters of a creek, heavily polluted with sewage, near Collingswood, N. J.

Summary

There is an unusual group of protozoa to be found in the oxygen-poor and oxygen-deficient waters of sewage treatment plants.

Most of these are apparently facultative anaërobes. A few appear to be obligatory anaërobes.

It is shown that the presence of dissolved oxygen in sewage allows *Opercularia* to thrive, while the absence of oxygen is fatal to this protozoön, if the condition endures for several days. The reverse conditions are found to obtain for *Trepomonas*, except that active aëration of the water is quickly fatal.

Six new species of protozoa from sewage or polluted waters are described.

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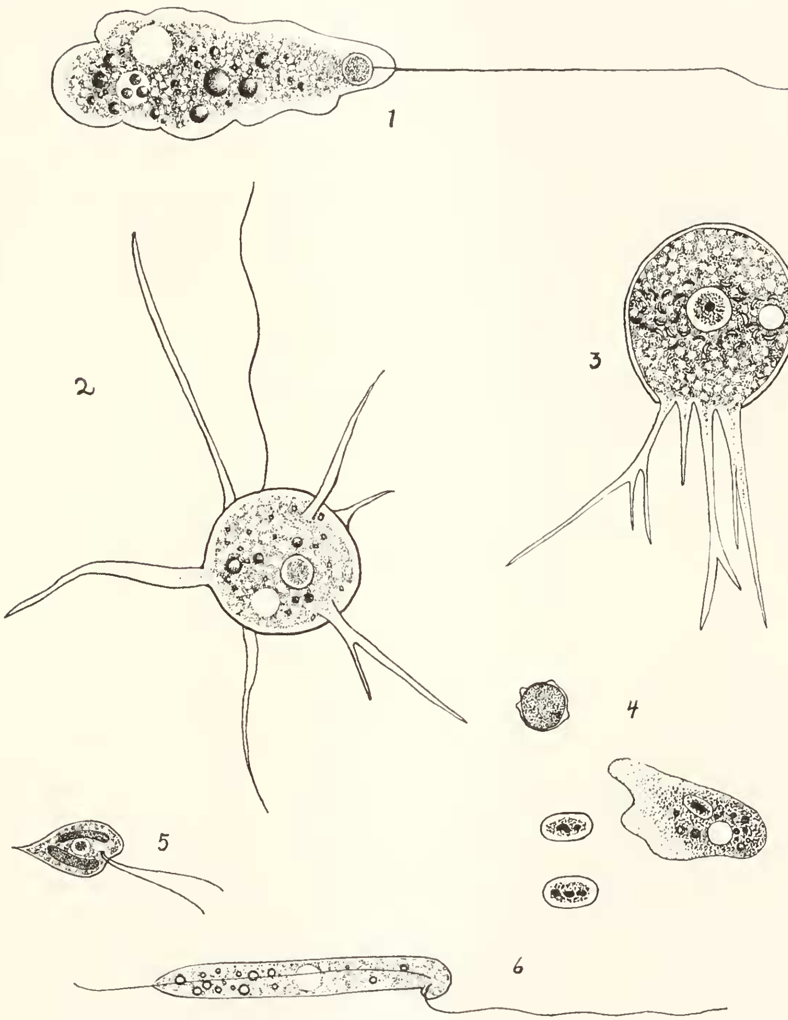

Plate I